Oxysterols: Chemical Synthesis, Biosynthesis and Biological Activities

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As a class of compounds, oxysterols have demonstrated a wide variety of biological properties. Due to the general interest in these compounds, new methods of chemical synthesis have been developed to provide them for biological investigation. The specific inhibition by oxysterols of cholesterol biosynthesis in mammalian cells has been shown to result primarily from a decrease in cellular levels of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity. Recent evidence suggests these cellular responses may be mediated by an oxysterol binding protein found in the cytosol of many lines of cultured cells. In certain instances, oxysterols have been shown to be produced in biological systems. These results support the supposition that oxysterols may regulate sterol biosynthesis at the cellular level. Included herein are the inhibitory effects of 9α, 11α-epoxycholest-7-en-3β-ol cholest-8-en-3β-ol-7-one and cholest-8-en-3β-ol-11-one on HMG-CoA reductase activity and their relative affinities for a cytosolic binding protein.
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Steroids bearing a second oxygen function in addition to that at carbon-3 have demonstrated a variety of diverse biological activities (1-3). Some of these include cytotoxicity, atherogenicity, carcinogenicity, mutagenicity, hypocholesterolemia and effects on specific enzymes. In addition, several oxysterols have been isolated from drugs used in folk medicine for the treatment of cancer (4-6). Other studies have shown that certain oxysterols have significant activity in the inhibition of DNA synthesis in cultured cells (7,8). A number of oxygenated derivatives of cholesterol and sterol intermediates in cholesterol biosynthesis have been found to be potent inhibitors of sterol biosynthesis in animal cells in culture (2,3,9). The specific inhibition of cholesterol biosynthesis in mammalian cells by oxygenated derivatives of cholesterol and lanosterol has been shown in many cases to decrease cellular levels of HMG-CoA reductase activity. This response has been attributed to a decreased rate of HMG-CoA reductase synthesis (9-11) and in some instances to an increase in enzyme degradation (9,11).

A large number of oxysterols have been evaluated for their abilities to repress HMG-CoA reductase activity in cultured mammalian cells (2,3,12). In general, potency has been found to vary over a wide range depending on the structural features of the oxysterol. As a general trend, inhibitory activity increases as the distance between carbon-3 and the second oxygen function becomes greater. Steroids with oxygen groups in ring D and the side chain have been shown to have the greatest activity. An intact side chain is a requirement for potent activity; a decrease in the length of the (iso-octyl) side chain results in decreased activity (13). Other noticeable trends indicate a relationship between inhibitory activity and the extent to which the second oxygen function is sterically hindered. In general, axial hydroxyl groups are more hindered and possess lower activities than the less hindered equatorial conformation (2,12,13). Steric hindrance from other parts of the steroid molecule also can result in diminished activity (i.e., effect of carbon-14 alkyl substituents on the carbon-15 hydroxyl group) (14). It has been suggested that oxygen functions in conformationally flexible positions such as those in ring D and in the side chain produce more inhibitory steroids due to increased effective hydrogen bonding or hydrophilic interactions with receptor molecules (3).

These observations suggest a regulatory mechanism which, by analogy to steroid hormone receptors and bacterial induction-repression systems, requires a binding protein to recognize oxysterols and mediate subsequent cellular events. Evidence for the existence of a specific cytosolic receptor protein for oxysterols has been presented (12,15). After the activities of a number of sterols were evaluated, a good correlation was found between the actions of certain oxysterols on HMG-CoA reductase in L cells and their affinity for a oxysterol binding protein (12).

Recently it has been shown that under certain conditions biological systems can be induced to produce oxygenated derivatives of cholesterol and lanosterol (16,17). These results add support to the hypothesis that oxysterols may be natural regulators of cholesterol biosynthesis in the intact cell (18). It has been suggested that such regulatory oxysterols may arise endogenously from cellular cholesterol by either nonenzymic or controlled enzymic oxidation of cholesterol or from the analogous oxidation of biosynthetic precursors of cholesterol (e.g., lanosterol) (3,18,19). An alternate and more interesting pathway requiring the formation of endogenous oxysterols as by-products of cholesterol biosynthesis has been described (16,17,20-23). Compounds such as 24,25-epoxylanost-8-en-3β-ol and 24,25-epoxycholest-5-en-3β-ol may be derived from squalene-2,3,22,23-dioxide (SDO). With the use of inhibitors of 2,3-oxidosqualene cyclase, an increased concentration of SDO can be induced. Upon removal of these inhibitors (4,4,10-trimethyl-*trans*-decal-3β-ol [21] or 3β - [2 - (diethyl-amino)ethoxy] androst -5 -en -17 - one [U18666A] [23]), SDO appears efficiently metabolized t polar products (21,23), presumably 24,25-epoxylanost-8-en-3β-ol and 24(*s*),25-epoxy-cholest-5-en-3β-ol (16,20,22). The metabolism of SDO was associated with a suppression of HMG-CoA reductase (17,23), indicating that cyclized derivatives of SDO have biological effects similar to oxysterols. The immediate cyclization product of SDO, 24,25-epoxylanost-8-en-3β-ol, and two related oxylanosterol analogs, lanost-8-ene-3β-25-diol and lanost-8-ene-25-ol-3-one, have been prepared by chemical synthesis (Fig. 1) and were found to be strong inhibitors of HMG-CoA reductase activity in cultured rat intestinal epithelial cells (24). These oxysterols caused a 50% inhibition of reductase activity at 0.85×10^{-7} M, 1.67×10^{-7} M, and 4.18×10^{-7} M, respectively.

Among the more interesting biological properties of certain oxysterols are their abilities to act as hypocholes-

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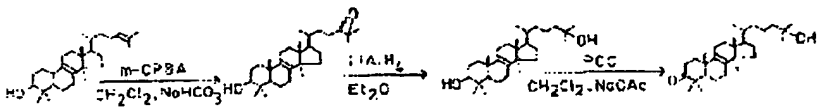

FIG. 1. Chemical synthesis of 24,25-epoxylanost-8-en-3β-ol, lanost-8-ene-3β-25-diol and lanost-8-ene-25-ol-3-one.

terolemic agents. Cholest-8(14)-en-3β-ol-15-one (25-28) and its palmitate and hemisuccinate esters (29), cholest-8(14)-en-3,15-dione (30) and 9α-fluorocholest-8(14)-en-3β-ol-15-one (31) have demonstrated sustained hypocholesterolemic activity in intact animals. The related unsaturated keto steroid cholest-4-en-3-one also is reported to possess similar activity (32). Recently two other oxysterols, cholest-8-en-3β-ol-7-one and cholest-8-en-3β-ol-11-one, were found to have significant but not sustained hypocholesterolemic activity when fed to rats at a level of 0.15% in the diet (Parish, E.J., Nandun, V.B.B., Seikel, J.M., Kohl, H.H., and Nusbaum, K.E., unpublished results). The action of these two oxysterols as inhibitors of HMG-CoA reductase and their affinity for a cytosolic binding protein are shown in Table 1.

Due to the potential importance and general interest in oxysterols as a class of compounds, new methods of chemical synthesis have been developed to provide them for biological investigations. Other approaches to chemical synthesis have included the streamlining of existing or "classical" methods of synthesis.

A number of 15-oxygenated sterols have been found to be potent inhibitors of HMG-CoA reductase and to actively suppress sterol synthesis in animal cells in culture. In addition, several 15-keto sterols and their derivatives have demonstrated significant hypocholesterolemic action when administered to intact animals (2,27,28). These results stimulated an extensive synthesis program of new 15-oxygenated sterols and intermediates salient in their preparation. Many of these compounds have been prepared from 3β-benzoyloxy-14,15α-epoxycholest-7-ene (33,34), a key intermediate in the synthesis of a large number of these compounds (2). A new approach to the synthesis of 15-oxysterols involving the chromium (VI) oxidation of the 8,14-diene system was utilized in a novel synthesis of the hypocholesterolemic agent 9α-fluorocholest-8(14)-en-3α-ol-15-one (35).

Several steroids with hypocholesterolemic properties have in common the α,β-unsaturated ketone functionality. New methodology has been developed to prepare these compounds from the corresponding allylic alcohol by selective oxidation using a pyridinium chlorochromate-amine reagent system (36-40). The unsaturated keto sterols cholest-8-en-3β-ol-7-one and cholest-8-en-3β-ol-11-one, cited previously for their hypocholesterolemic activity, have been prepared from the key intermediate 3α-benzoyloxy-9α,11α-epoxycholest-8-ene by a modification of known synthetic methods (Fig. 2) (41-46).

Among the most frequently encountered oxysterols are those with a keto or hydroxyl function at carbon-7. These compounds have been found in animal tissues and foodstuffs (1) and certain folk medicines (4-6,47) and have been shown to be significant inhibitors of HMG-CoA reductase (2,12), sterol synthesis (2,48) and cell replication (49-51). Recently a synthetic sequence was developed for the synthesis of 4,4'-dimethyl-7-oxygenated sterols using commercial cholesterol as a starting material (52). The concentrations of those sterols required for 50% inhibition of HMG-CoA reductase activity were similar to those reported for the corresponding sterols devoid of the 4,4'-dimethyl functionality (Table 1).

In the lanosterol series, a simplified method for the preparation of 14α-hydroxymethyl derivatives of 24,25-dihydrolanosterol has been described (53). These compounds are proposed intermediates in the biosynthesis of cholesterol from lanosterol and were found to be potent inhibitors of HMG-CoA reductase and sterol biosynthesis (54). The previously described oxylanosterol derivatives 24,25-epoxylanost-8-en-3β-ol, lanost-8-ene-3β-25-diol and lanost-8-ene-25-ol-3-one have been prepared (Fig. 1) directly from commercial lanosterol (a mixture of lanosterol and 24,25-dihydrolanosterol) in a simplified synthesis. The main feature of this synthesis is the direct monoepoxidation of lanosterol with *m*-chloroperbenzoic acid to yield the 24,25-epoxide. This material was isolated by selectively removing the remaining commercial starting material during repetitive crystallization from methylene chloride-

TABLE 1

Oxysterol Repression of HMG-CoA Reductase and Relative Affinity for Oxysterol-Binding Protein

Sterol	Repression of HMG-CoA reductase[a]	Relative binding affinity[a]
3β-Hydroxy-4,4'-dimethylcholest-5-en-7-one	1.5	—
4,4'-Dimethylcholest-5-ene-3β,7α-diol	1.5	—
4,4'-Dimethylcholest-5-ene-3β,7β-diol	1.7	—
3β-Hydroxycholest-5-en-7-one	1.7	1.4
Cholest-5-ene-3β,7α-diol	2.5	1.1
Cholest-5-ene-3β,7β-diol	2.7	4.4
9α,11α-Epoxycholest-7-en-3β-ol	2.3	3.8
3β-Hydroxycholest-8-en-7-one	1.1	0.49
3β-Hydroxycholest-8-en-11-one	9.0	-

[a]The values given are the concentrations necessary for 50% response in each assay calculated as described in the Experimental section.

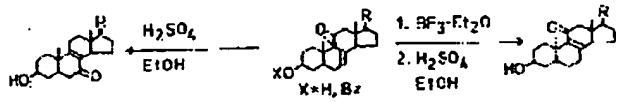

FIG. 2. Chemical synthesis of cholest-8-en-3β-ol-7-one and cholest-8-en-3β-ol-11-one.

methanol, followed by liquid chromatography of the remaining noncrystalline portion (24; Panini, Sexton, Parish and Rudney, submitted for publication). Epoxide ring opening with hydride resulted in the carbon-25 alcohol. Oxidation of the 3β-hydroxyl group with pyridinium chlorochromate resulted in the 3-keto derivative (55).

Research into the chemistry and biochemistry of oxysterols has resulted in major developments summarized briefly in this report. Further extension of the scope of this research is anticipated, especially in understanding the mechanism(s) of possible oxysterol regulation of mammalian cholesterol biosynthesis.

EXPERIMENTAL

The sterols 9α,11α-epoxycholest-8-en-3β-ol, cholest-8-en-3β-ol-7-one and cholest-8-en-3β-ol-11-one were prepared by chemical synthesis (to be presented elsewhere) and were found to have a purity of 98% or greater by gas liquid chromatography and/or thin layer chromatographic analyses (39).

The experimental procedures used in cell culture studies using mouse L cells (a subline of NCTC clone 929 mouse fibroblasts) and the determination of HMG-CoA reductase in cell homogenates have been described previously (12,52). The concentration of sterol in the medium that gave a 50% repression of HMG-CoA reductase after five hr of incubation was determined graphically from a plot of inhibitory activity (percentage of the control value) vs at least five concentrations of sterol. The assay of relative binding affinity of unlabeled sterols by competition with 25-hydroxy[^{3}H] cholesterol with the cytosolic binding protein has been described previously (12).

RESULTS AND DISCUSSION

The oxysterols 9α,11α-epoxycholest-7-en-3β-ol, 3β-hydroxycholest-8-en-7-one and 3β-hydroxycholest-8-en-11-one have been prepared by chemical synthesis using established methods (Fig. 2) (41-46). These compounds contain a diversity of structure in rings B and C; therefore it seemed worthwhile to examine them for their abilities to inhibit HMG-CoA reductase and their affinities for a cytosolic oxysterol-binding protein (Table 1, Fig. 3).

The epoxy sterol 9α,11α-epoxycholest-7-en-3β-ol was found to be a potent inhibitor of HMG-CoA reductase in L cells in culture and also exhibited moderate affinity of the oxysterol binding protein. This is the first example of a 9α,11α-epoxide to be examined in these systems. The keto sterol 3β-hydroxycholest-8-en-7-one shows excellent inhibition of reductase and a strong affinity for the oxysterol binding protein. Oxysterols with a ketone or hydroxyl function at carbon-7 are among the most frequently encountered. The results obtained from other carbon-7 oxysterols are presented in Table 1 for comparison (12,52). The oxysterol 3β-hydroxycholest-8-en-11-one was found to be a poor inhibitor of HMG-CoA reductase. This result was somewhat unexpected due to potent inhibition shown by another carbon-11 oxygenated sterol, cholest-7-ene-3β,11α-diol, which showed a 50% reduction of reductase activity at a 0.55 μM concentration (12). In addition, the keto sterol had no detectable affinity for the oxysterol binding protein, a property consistent with its poor ability to inhibit reductase activity.

The results described here are an addition to the knowledge of the correlation of sterol structure and the repression of HMG-CoA reductase activity and relative binding affinities for the cytosolic oxysterol-binding protein. The results of these and many other studies may aid in understanding the role of oxysterols in the regulation of sterol synthesis.

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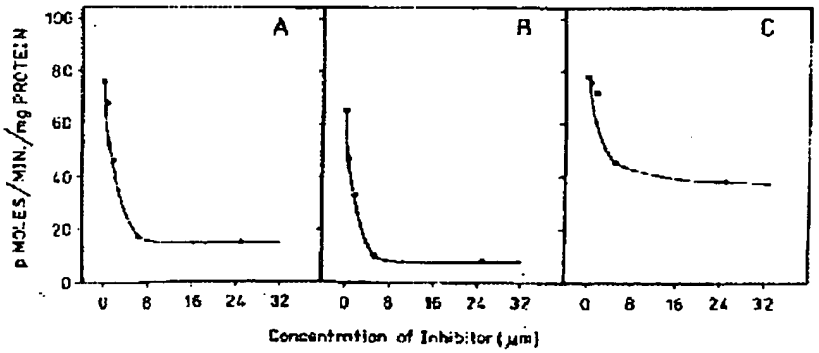

FIG. 3. Effects of oxysterols on the level of HMG-CoA reductase activity in L cells. A, 9α,11α-epoxycholest-7-en-3β-ol; B, 3β-hydroxycholest-8-en-7-one; C, 3β-hydroxycholest-8-en-11-one.

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